

## INHIBITION IN THE EYE OF LIMULUS

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(Received for publication, October 7, 1955)

The integration of nervous activity in sensory systems often begins in the receptor organs themselves. Numerous electrophysiological studies of the vertebrate visual system, in particular, have shown that in the eye the activity of elements in one region of the retina may be affected by illumination of other regions. Both excitatory and inhibitory interactions have been demonstrated. Granit's extensive researches, especially, have shown the importance of these retinal processes in visual physiology. (For reviews, see Granit, 1947, sections I and II, and 1955, chapter 2.) Interaction in the vertebrate eye may be ascribed to the complex organization of the retina, which is indeed a "nervous center."

In the lateral eye of the horseshoe crab, *Limulus polyphemus* (L.), the histological organization is much simpler than in the vertebrate retina. Nevertheless, the sensory elements in this eye do exert an influence upon one another (Hartline, 1949). The interaction is inhibitory: the frequency of the discharge of impulses in a single optic nerve fiber is decreased and may even be stopped by illuminating areas of the eye in the neighborhood of the sensory element from which the fiber arises. The occurrence of a purely inhibitory action in a relatively simple eye is of general interest; the role that may be played by inhibitory interaction in enhancing contrast gives it an importance to visual physiology. It is the purpose of this paper to report in detail our experiments on this inhibitory phenomenon in the eye of *Limulus*.

### *Material*

The lateral eye of *Limulus* is a coarsely faceted compound eye containing, on the average, some 800 ommatidia. In a medium sized adult (25 cm. broad) each eye forms an ellipsoidal bulge on the side of the carapace, about 12 mm. long by 6 mm

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\* National Research Council Fellow in the Biological Sciences during part of the period covered by this research (1950-51).

† Much of the experimental work in this study was done in the Jenkins Laboratories of Biophysics, Johns Hopkins University, Baltimore, supported by contract Nonr248(11) with the Office of Naval Research. Reproduction in whole or in part is permitted for any purpose of the United States Government.

wide. Each ommatidium has an optical aperture about 0.1 mm. in diameter; the facets are spaced approximately 0.3 mm. apart, center to center, on the surface of the eye. Each ommatidium has a "visual field" subtending a few square degrees of solid angle about its optical axis. Light coming from within this solid angle reaches the sensory component of the ommatidium directly through the transparent cornea and the crystalline cone of the ommatidium. The optical axes of the ommatidia diverge, so that the visual fields of all those in one eye (each one overlapping somewhat those of its neighbors) cover approximately a hemisphere. The location of the visual field of each ommatidium within the visual field of the entire eye is correlated in a more or less regular manner with the position of that ommatidium on the surface of the eye (*cf.* Waterman, 1954, for a recent study of the directional sensitivity of the ommatidia of the lateral eye of *Limulus*).

The sensory component of each ommatidium consists of a cluster of cells that give rise to nerve fibers (*cf.* Hartline, Wagner, and MacNichol, 1952). There are two kinds: 10 to 20 retinula cells arranged radially about the axis of the ommatidium, and one eccentric cell (occasionally two). The axons of these cells leave the heavily pigmented envelope of the ommatidium as a small bundle, and most, perhaps all, of them proceed more or less directly and apparently without interruption to become fibers of the optic nerve.

Immediately after emerging from the ommatidia the axons of the retinula and eccentric cells become associated with an extensive system of cross-connecting strands of nerve fibers, to form a three dimensional network about 1 mm. thick (Fig. 1). The organization of this "plexus" (Watase, 1890) is not fully understood. No nerve cell bodies have been observed within it and it has not yet been possible to determine with certainty the origins and terminations of the fibers that make up the cross-connecting strands. However, it has been observed that the plexus fibers come into close association with the axons of the eccentric cells where they emerge from the proximal ends of the ommatidia. The plexus clearly furnishes numerous anatomical interconnections among the ommatidia.

Each ommatidium apparently functions as a single "receptor unit" in the discharge of optic nerve impulses. The action potentials recorded from a large bundle of fibers dissected from the optic nerve give evidence of the activity of many fibers when a large area of the eye is illuminated, but when the illumination is carefully confined to one ommatidium, a regular train of uniform action potential spikes is elicited, typical of the activity of just one single fiber (see also Waterman and Wiersma, 1954). If the bundle is dissected until but a single active fiber is left, a discharge of impulses can be elicited in it by illumination of one, and only one, particular ommatidium. The strand of optic nerve containing such a single active fiber can be followed in the peripheral direction through the plexus by dissecting it free of surrounding tissue, all the way to the ommatidium in which its activity originates.

The discharge of impulses may be recorded directly from an ommatidium by means of a micropipette electrode, but only from a sharply localized region within the receptor structure (Hartline, Wagner, and MacNichol, 1952). In recent experiments, done in collaboration with Dr. MacNichol, we have been able to see the living eccentric cells in exposed ommatidia; upon thrusting a micropipette electrode into one of them, large action potential spikes have been observed, and from no

other structure in the ommatidium could such spikes be recorded. Apparently the discharge of impulses recorded in a single fiber of the optic nerve originates in the eccentric cell of the ommatidium from which that fiber comes. Waterman and Wiersma (1954) have reached the same conclusion. The function of the retinula cells and their associated fibers is still unknown.

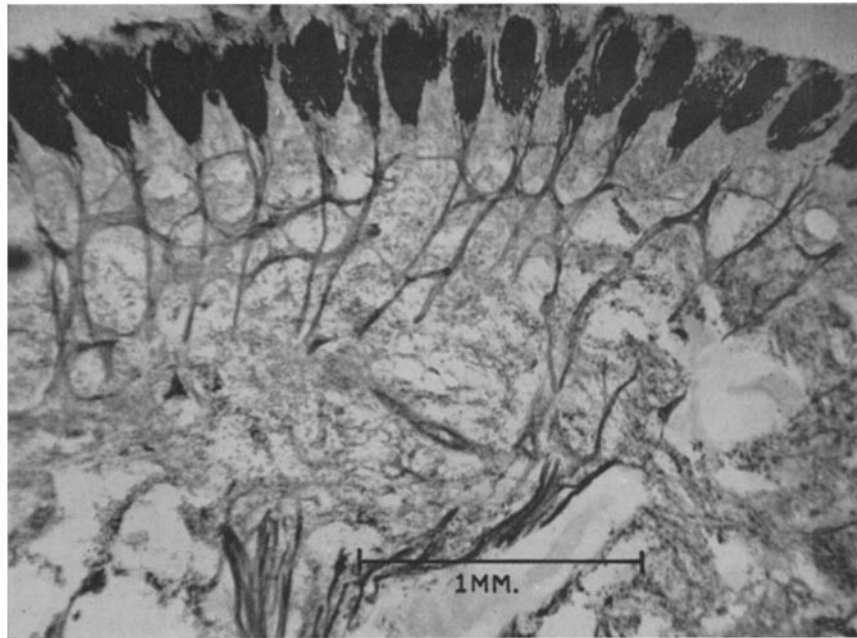


FIG. 1. Section through part of a lateral eye of an adult *Limulus*, perpendicular to the cornea, showing the heavily pigmented portions of the ommatidia (upper border of the section), the bundles of nerve fibers emerging from them, the plexus of interconnecting fibers, and a portion of the optic nerve (bottom of figure). The chitinous cornea with the attached crystalline cones of the ommatidia had been stripped away prior to fixation. Samuel's silver stain. (Prepared by W. H. Miller.)

The axons of the eccentric cells apparently traverse the plexus without interruption by one way synapses, for Prof. T. Tomita, working in our laboratory, observed that antidromic impulses elicited by electrical stimulation of a single fiber dissected from the optic nerve could be recorded by a microelectrode placed on the ommatidium in which that fiber originated.

#### Methods

In most of the experiments reported in this paper, we recorded the action potentials of single fibers dissected from the optic nerve. A lateral eye was excised with 1 to 2 cm. of optic nerve and mounted in a chamber maintained at 18°C. A small

strand containing only a single active fiber was separated from the nerve. Its proximal end was lifted out of the sea water or blood bathing the back of the eye, placed over wick electrodes, and its action potentials amplified and recorded oscillographically (Hartline and Graham, 1932). The ommatidium from which this fiber arose was stimulated by focussing a small spot of light directly upon its facet; receptors in other regions of the eye were then illuminated to determine the effect of their activity upon the responses of this particular ommatidium.

In a few experiments (specifically indicated) we recorded action potentials within an ommatidium by means of a microelectrode. A glass pipette electrode (tip  $\frac{1}{2}$   $\mu$  to 3  $\mu$ ), filled with sea water, was thrust into one of the ommatidia to record the nerve impulses at the site of their origin in the receptor unit. This was done either by stripping away the cornea of the eye and thrusting the electrode axially into the exposed distal end of an ommatidium (method devised in collaboration with Mr. M. Wolbarsht), or by cutting the eye in a plane perpendicular to the cornea, and thrusting the electrode into the side of one of the ommatidia lying near the cut edge (Hartline, Wagner, and MacNichol, 1952).

The apparatus for illuminating the eye (*cf.* Hartline and McDonald, 1947) provided two independently controllable beams of light from a common source (incandescent tungsten filament). The intensities of these beams could be varied by optical wedges of neutral density calibrated in place; the exposures were controlled by electromagnetic shutters operated by electronic timing devices. In most experiments the pattern of illumination of each beam was formed by an aperture in a diaphragm, an image of which was focussed on the corneal surface of the eye by a photographic objective lens. The preparation was mounted upon a horizontal turntable, and the beams were directed upon it by adjustable mirrors, so that the light could be made to fall upon the eye from any direction necessary to produce an optimum effect.

Several arrangements of the beams were required. In many of the experiments the two beams were combined by means of a semireflecting mirror so that their fields of illumination were superimposed. The beams then could illuminate, in common, a circular region of the eye 5 mm. in diameter; diaphragms in each beam were used to limit the illumination to the desired receptors within this region. Thus, for example, a small spot of light 0.1 to 0.2 mm. in diameter, formed by a diaphragm in one beam, could be centered on the corneal facet of an ommatidium from which a discharge of nerve impulses was to be recorded; a diaphragm in the other beam could then be adjusted to form another spot of light on a nearby region of the eye to inhibit this discharge. The size and shape of this second spot and its location with respect to the first one could be varied as desired within the limits imposed by the 5 mm. field available to the two beams.

In some experiments it was necessary to illuminate regions of the eye separated by more than 5 mm. or to control independently the angles of incidence of the beams. We then directed the beams through separate mirror systems, thus enabling each one to be brought onto any part of the eye from the appropriate direction for maximal stimulating effectiveness of the ommatidia it illuminated. In addition to the main optical system just described, a small accessory light source and projector were sometimes used in special experiments.

## RESULTS

*1. General Properties of the Inhibition*

Illumination of regions of a *Limulus* lateral eye in the vicinity of any particular ommatidium reduces the ability of that receptor unit to discharge impulses in response to light. During such illumination, the threshold of the receptor unit is raised, the number of impulses it discharges in response to a suprathreshold flash of light is diminished, and the frequency with which it discharges impulses during steady illumination is reduced. The latter effect is especially convenient for demonstrating the properties of the inhibition.

In an illustrative experiment, an ommatidium from which discharges of impulses were recorded was illuminated steadily by a small spot of light focussed upon its facet. After this exciting light had been shining for several seconds, to permit the frequency of the discharge to reach a steady level, a region of the eye surrounding the selected ommatidium was illuminated. The effect on the discharge is shown in Fig. 2, upper record. When the surrounding light was turned on, the discharge suddenly underwent a drop in frequency (from 65 impulses per second), then recovered partially and continued at a lower rate (approximately 30 per second) as long as the region surrounding the ommatidium was illuminated. When this inhibiting light was turned off, the frequency rose to its original value. At the onset of the inhibiting illumination, there was a latency of 0.14 second before the frequency began to fall; at the end of the illumination it took about 0.3 second to recover its original value.

This record shows the typical features of the inhibition of nervous activity of a receptor unit that results from stimulation of other sensory elements near it in the eye. A sudden onset of the inhibition after an appreciable latency, a deep initial minimum in frequency, a steady maintenance of a depressed rate of discharge, and a prompt though not instantaneous recovery after turning off an inhibiting light are characteristic. During inhibition the discharge is neither more nor less regular, as a rule, than the discharge at a comparable frequency obtained in response to a weaker stimulating light in the absence of inhibition. The level of frequency can be graded smoothly by varying the factors that affect the degree of inhibition; there is no suggestion that the depression of frequency is brought about by the dropping out of impulses from the regular series of an uninhibited discharge. The return of the frequency to the uninhibited level usually takes place directly, although in some preparations a slight "overshoot" has been observed (*cf.* Fig. 2, lower record, and Fig. 4, "0.0").

Inhibition is exhibited not only when the activity is recorded from the fibers of the optic nerve but also when the impulses are recorded close to

their point of origin in the ommatidium. Fig. 2, lower record, is an oscillogram obtained in a typical experiment in which a microelectrode was thrust into an ommatidium to record the action potential spikes from its eccentric cell. This ommatidium was excited by shining a small beam of light into it; illumination of a region of the eye close to it then produced a slowing of the

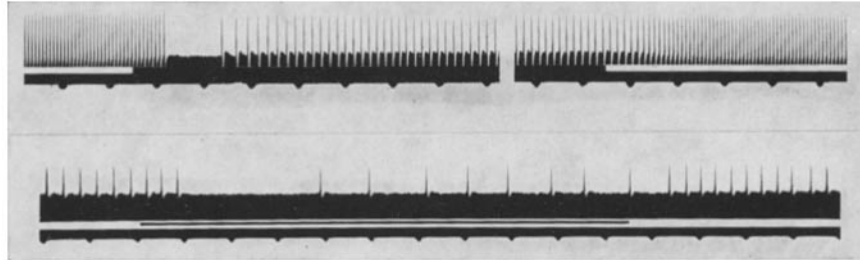


FIG. 2. Oscillograms of electrical activity of single receptor units in the lateral eye of *Limulus*, showing the reduction in frequency that occurred when regions of the eye were illuminated near these units.

In each of the two experiments shown, a small spot of light, projected on the facet of the ommatidium in which the activity originated, had been turned on several seconds before the beginning of the record. During the interval marked by the signal (blackening of the white band above the time marks) a region of the eye was illuminated near the ommatidium under observation. Amplifier time constant 0.1 second. Time in one-fifth seconds.

Upper record: Action potentials from a single fiber dissected from the optic nerve. The inhibiting illumination covered an annular region surrounding the facet of the ommatidium in which that optic nerve fiber originated. The inner boundary of the annulus was approximately 2 mm. in diameter, the outer boundary approximately 4 mm. in diameter.

Lower record: Action potentials recorded by a microelectrode thrust into the distal end of an ommatidium after removal of the cornea and crystalline cone. The inhibiting illumination was a spot of light approximately 2 mm. in diameter, centered about 2 mm. from the ommatidium in which the activity was being recorded.

discharge rate, similar to that observed when the sensory discharge was recorded from a fiber dissected from the optic nerve. The inhibitory influence is evidently exerted within the ommatidium itself, upon some process that determines the rate of discharge of nerve impulses.

Illumination of regions of the eye in the neighborhood of any particular ommatidium not only reduces the ability of that receptor unit to discharge impulses in direct response to light but also inhibits activity that can occur when it is in complete darkness. The after-discharge following intense stimulation by light, the spontaneous activity exhibited by some preparations in

complete darkness, and activity that can be induced by an excess of  $K^+$  in the solution bathing the eye have all been observed to be inhibited by illuminating a region of the eye close to the discharging ommatidium, even though that receptor unit was in darkness when the inhibiting light was turned on.

In any one eye in which marked inhibition was observed, it was our experience that every single fiber picked at random from the optic nerve showed some degree of reduction in the frequency of its discharge when an appropriate region of the eye was illuminated near the ommatidium from which it originated. We have often prepared three or four, sometimes even more, single fibers in the course of an experiment on one eye, coming from ommatidia in different regions of the eye, all of which showed typical inhibitory effects. The same has been found with a microelectrode, testing many ommatidia in succession.

Simultaneous observation of the responses from two receptor units has shown that nearby ommatidia often inhibit one another mutually. An example is given in Fig. 3. In this experiment the discharges of impulses in two optic nerve fibers were recorded at the same time; the responses may be distinguished in the records by differences in the size and shape of the action potential spikes. Fibers were chosen that came from two ommatidia close together in the eye, each illuminated by a separate spot of light confined to its facet. To obtain the upper record one ommatidium (giving rise to the large spikes) was illuminated for several seconds until its discharge had reached a steady frequency. The second ommatidium was then illuminated, initiating a train of impulses (small spikes) in its fiber, and at the same time slowing the discharge from the first receptor unit. To obtain the lower record, the roles of the two ommatidia were interchanged; inhibition of the discharge from the second ommatidium resulted when the first one was illuminated. It is evident that these two individual receptor units in the eye inhibited each other mutually. Many other experiments have shown that mutual inhibition is common between ommatidia that are close together in an eye, although it has been observed that the effects are not always equally strong in the two directions. The consequences of such interaction are complex, and we will defer their treatment to a later communication (*cf.* Hartline, Wagner, and Tomita, 1953, and Hartline and Ratliff, 1954).

The inhibitory influence appears to be mediated by the plexus of fibers that lies behind the layer of ommatidia. In many experiments a single fiber was picked up from the optic nerve and then isolated by dissection all the way up to the ommatidium in which it originated (the isolated fiber was always kept immersed in the blood bathing the preparation, except for its proximal end, which was placed on the electrodes). During the course of

such dissection, the inhibitory effect diminished progressively as the fiber bundle was cut away from its connections within the plexus. Sometimes cutting a prominent cross-connection seemed to be especially effective in reducing the inhibitory effect that could be obtained. When the dissection had been extended up to and completely around the pigmented body of the ommatidium, no inhibition could ever be obtained by illuminating adjacent

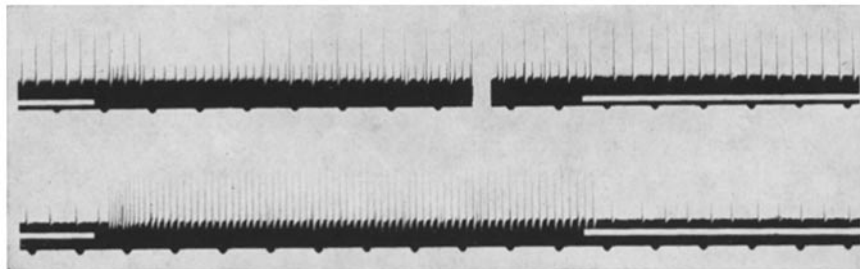


FIG. 3. Mutual inhibition of two receptor units in the eye of *Limulus*. Activity was recorded from an optic nerve strand containing two active fibers. The responses of the two fibers may be distinguished by the different sizes of their action potential spikes. The ommatidia from which these fibers arose were 0.7 mm. apart; each was illuminated independently of the other by a small spot of light confined to its facet. For the upper record, the ommatidium whose optic nerve fiber gave the large spikes was illuminated steadily, beginning 5 seconds before the start of the record; the signal marks the period of illumination of the other ommatidium, whose fiber gave the small spikes. The record shows the slowing of the rate of discharge of the large spikes, shortly after the onset of the discharge of small ones, and recovery to the original frequency after the small ones stop.

For the bottom record the roles of the two units were interchanged: the frequency of the small spikes was decreased during the discharge of the large ones. (Two small spikes were discharged during the first 0.2 second of the discharge of large ones; there followed a gap of 0.5 second, after which the discharge of small spikes was renewed at a frequency lower than that which occurred before and after the discharge of large ones.)

regions of the eye. These observations strongly suggest that the inhibitory effect depends on the integrity of nervous pathways in the plexus. Nevertheless, more extensive experimentation on this crucial point is still desirable.

The inhibitory effects that we have described are typical of those observed in most of several hundred lateral eyes<sup>1</sup> of *Limulus* that we have studied. Occasionally preparations were found that showed only weak inhibition. In a very few preparations the inhibitory effect diminished, became sluggish,

<sup>1</sup> Dr. W. H. Miller, in our laboratory, has observed similar inhibitory effects in the median eyes of this animal.



and sometimes became imperceptible several hours after excision of the eye, even though the optic nerve discharges in response to light were undiminished.

Effects that might be attributed to the physiological spread of excitatory influences in the eye of *Limulus* have never been observed. However, the physical scatter of light in the optical system and within the cornea of the eye itself sometimes complicates the manifestation of inhibition caused by illuminating regions close to an ommatidium. In some preparations the sudden gap in the discharge that marked the onset of inhibition was preceded by a very brief rise in frequency—two or three intervals between impulses that were shorter than the preceding ones in the regular series. Correspondingly, when the inhibiting spot of light was turned off, a very brief drop in frequency occurred before the recovery from the inhibition began. These fluctuations could be traced to the direct excitatory effects of stray light from the inhibiting beam scattered into the ommatidium under observation, which provided an increment to its stimulus, and which acted with a short latency (cf. MacNichol and Hartline, 1948). Such effects could always be reduced, and often completely abolished, by using a separate optical system for the inhibiting beam. Sometimes it was necessary to shave away as much as possible of the chitinous cornea to reduce light scatter within the eye. When the effects produced by stray light had been abolished by such procedures, no evidence for excitatory interaction could be observed. We believe that the physiological interaction in the eye of *Limulus* is purely inhibitory.

## 2. Factors Affecting the Magnitude of the Inhibition

The degree of inhibition of the response of a receptor unit by illumination of regions of the eye in its neighborhood may be measured by taking the difference between the frequency of impulses discharged during a period of inhibiting illumination and the frequency during a comparable interval of time in a control exposure, when no light is shining on the neighboring region. The magnitude of the inhibition depends upon a number of factors. Those that we will describe in this paper are (a) the intensity of the inhibiting illumination; (b) the area of the region covered by the inhibiting illumination; (c) the location of this region with respect to the ommatidium under observation, and (d) the level of excitation of this ommatidium.

(a) *Effect of Intensity of Inhibiting Illumination.*—The degree to which the activity of an ommatidium is inhibited by illumination of regions of the eye near it is greater the higher the intensity of that illumination.

This is shown in the experiment illustrated in Fig. 4, in which each graph shows the time-course of the frequency change when an inhibiting light was turned on during steady illumination of an ommatidium. The different graphs are from records taken with different intensities of inhibiting illumination.

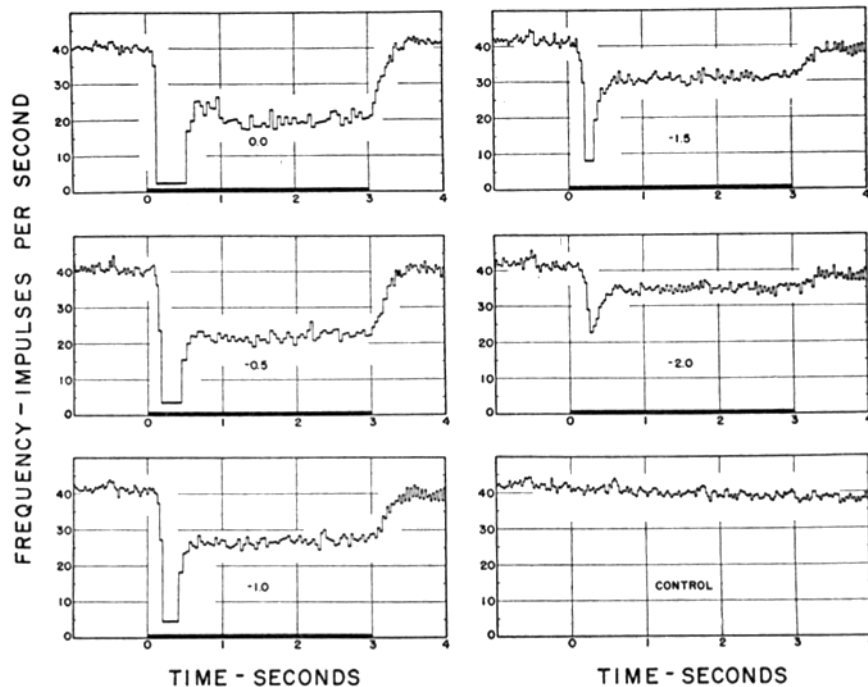


FIG. 4. Inhibition of the discharge of impulses in a single optic nerve fiber by various intensities of illumination on a region of the eye near the ommatidium from which the fiber arose. Frequency of impulse discharge is plotted as ordinate *vs.* time as abscissa. The graphs were prepared from oscillograms similar to those of Fig. 2: a horizontal line has been plotted for each interval between successive impulses, beginning at the abscissa of one impulse and ending at that of the next, having an ordinate equal to the reciprocal of the time interval between the two impulses. The ommatidium from which the fiber arose was illuminated steadily for 10 seconds by a small spot (0.2 mm. diameter) of light of fixed intensity (0.2 lumen/cm.<sup>2</sup> on the eye), starting 4 seconds before the onset of the inhibiting light, which illuminated a region of the eye 1 mm. in diameter, centered approximately 1.5 mm. from the ommatidium. The inhibiting light was turned on for a period of 3 seconds, as indicated by the heavy line at the base of each graph. The intensity of the inhibiting illumination is indicated on the respective graphs by the numbers, which are logarithms of relative intensity values. The most intense illumination ( $\log I_{\text{inhib.}} = 0.0$ ) was 0.5 lumen/cm.<sup>2</sup> on the surface of the eye. The "control" was taken from a comparable record, for which no inhibiting light was turned on. Temperature 18°C.

The more intense the inhibiting illumination, the deeper and longer was the initial depression of the frequency of the discharge and the greater was the depression of the steady level that was reached after the inhibiting light had been shining for a second or more. The quantitative relation between

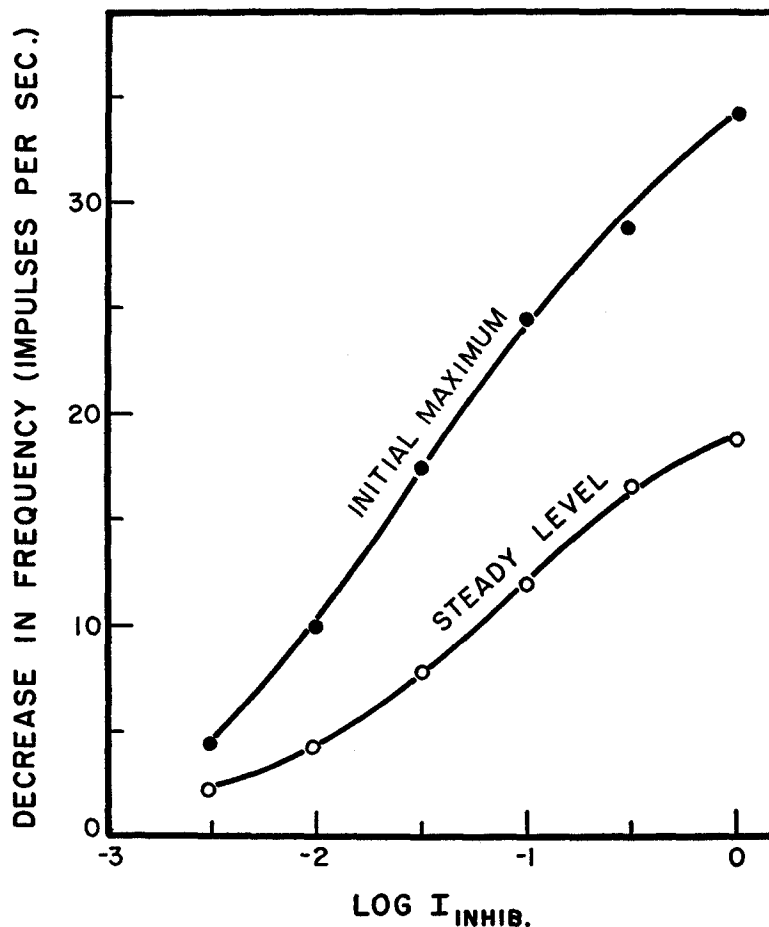


FIG. 5. Relations between intensity of inhibiting illumination and the magnitude of initial maximum and subsequent steady level of inhibition. From the experiment of Fig. 4. The lower curve (open circles) shows the decrease in average frequency measured over the second and third seconds of inhibiting illumination (determined by subtracting the frequency of the inhibited discharge from the average frequency measured over a comparable control period). The upper curve (solid circles) shows the decrease similarly measured in each of the records over that half-second period which exhibited the lowest average frequency (*i.e.*, maximum decrease in frequency).

intensity of inhibiting light and depression of frequency it produced is shown in Fig. 5. The upper curve refers to the initial maximum depression, the lower curve to the steady level of the depression. The inhibiting effect, measured by the depression in frequency of a steadily excited receptor unit, varied approximately linearly with the logarithm of the intensity of the illumination on neighboring receptors.

(b) *Effect of Areal Extent of Inhibiting Illumination.*—The magnitude of the inhibition exerted upon the response of an ommatidium depends not only upon the intensity of illumination on a region of the eye near it, but also upon the number of facets covered by this illumination: the larger the area of the eye illuminated by the inhibiting beam, the greater is the slowing of the rate at which the ommatidium discharges nerve impulses.

This is shown in an experiment in which annular patterns of light of constant intensity were projected onto the surface of the eye, surrounding the facet of the ommatidium giving rise to the nerve fiber that was on the recording electrodes. Different areas of the annulus were obtained by enlarging its outer boundary. The greater the area, the greater was the depression of frequency of the discharge from this ommatidium, which was independently illuminated at a constant intensity (Fig. 6). It is clear that the receptors in the regions of the eye surrounding the ommatidium contributed inhibitory influences that were summed in determining the total inhibition of its response; the greater the number of neighboring receptors stimulated, the greater was their inhibitory effect.

As shown in Fig. 6, the reduction in frequency was not in a simple proportion to the area of the eye illuminated. Several factors probably influenced the quantitative relation in this experiment. As the outer diameter of the annulus was increased, the additional ommatidia illuminated probably exerted smaller inhibitory influences upon the central ommatidium because of their increased distance from it; also, these receptors were undoubtedly stimulated less effectively than were those at the inner border of the annulus because of the increased divergence of their optical axes with respect to the direction of the incident light. Perhaps the most important factor, however, was the mutual inhibitory interaction among the receptor units involved (*cf.* Hartline and Ratliff, 1954). We will defer the treatment of the exact law of spatial summation of the inhibitory influences to a later report.

(c) *Effect of Location of Inhibiting Illumination.*—The response of an ommatidium is most effectively inhibited by illumination of other ommatidia located close to it; the effectiveness diminishes with increasing distance. Usually, however, some degree of inhibition of an ommatidium can be produced by illumination anywhere within a region surrounding it that may cover as much as one-half of the total area of the eye.

The variation in the magnitude of inhibition produced by illumination of regions of the eye located at different distances from an ommatidium is illustrated in the following experiment. The discharge of impulses was recorded from an ommatidium situated near the center of the eye. This ommatidium was illuminated by a small accessory optical projector, adjusted for optimal stimulating effectiveness. This projector was mounted on the turn-

table that carried the preparation, so that once correctly adjusted it remained in a fixed position with respect to the eye. Another beam, for inhibiting the

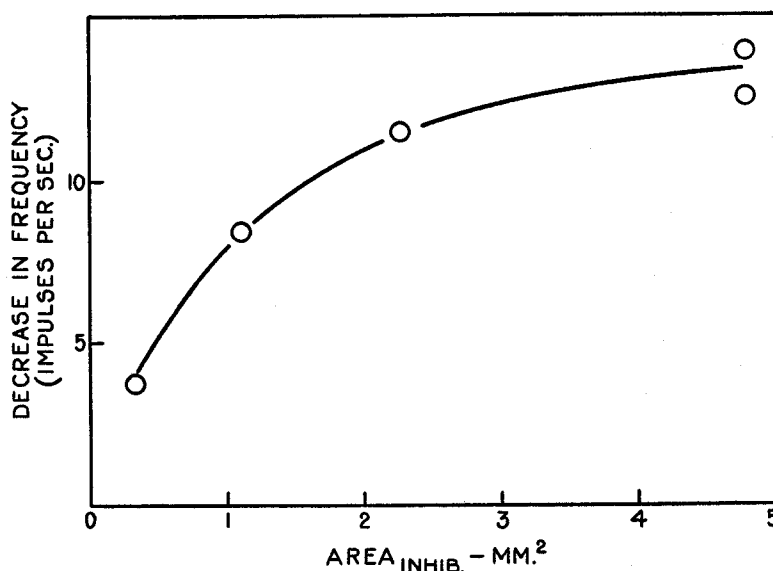


FIG. 6. The relation between the magnitude of inhibition (decrease in frequency) and the area ( $\text{mm}^2$ ) of the region on the surface of the eye illuminated by an inhibiting beam of fixed intensity. Records were obtained of the action potential spikes in a single optic nerve fiber from an ommatidium illuminated for 10 seconds by a small spot of light (0.25 mm. diameter and of fixed intensity) focussed on its facet. The inhibiting light illuminated an annular region concentric with the small spot. Four annular patterns having different areas were tested. The inner diameter of the annulus was kept at 0.38 mm.; its outer diameter was 0.75, 1.25, 1.75, or 2.50 mm. The inhibiting light was turned on for 3 seconds, beginning 4 seconds after the onset of the illumination on the ommatidium from which responses were recorded. The decrease in frequency was determined by measuring the average frequency of discharge over the second and third seconds of inhibiting illumination and subtracting this value from the estimated control frequency. The latter was obtained by interpolation between the average frequency of the uninhibited discharge occurring just before and that occurring just after the inhibiting illumination. (Illumination on the surface of the eye was approximately 20 lumens/ $\text{cm}^2$  for the small central spot, 2 lumens/ $\text{cm}^2$  for the annulus.)

activity of the ommatidium, was directed onto the eye from the main optical system. The spot formed by this beam could be moved to various locations on the surface of the eye, and the direction of incidence adjusted for maximum inhibiting effect at each location. This inhibiting spot was 1 mm.

in diameter and of fixed intensity. The stimulating light was turned on for a period of 8 seconds; during the last 5 seconds of this exposure the inhibiting light was turned on and the impulses discharged during this 5 second interval were counted. This count was compared with the number of impulses discharged in a comparable interval during a control exposure, when only the stimulating light was shining.

When the inhibiting spot was centered at a distance of 1 mm. from the ommatidium, it decreased the number of impulses discharged in 5 seconds by 50 (from 110 to 60); after it had been moved to a location 3 mm. away, the maximum effect it could produce at the intensity employed was to decrease the number of impulses discharged in 5 seconds by only 8. Moved to a distance of 5 mm., it had no perceptible effect at this intensity, no matter how the direction of incidence of the beam was adjusted. The regions tested in this part of the experiment were located on a line extending lengthwise of the eye (antero-posterior). At right angles to this direction (dorso-ventral) the inhibitory effectiveness diminished more rapidly with increasing distance, no measurable effect being produced when the spot was only 2.8 mm. from the ommatidium under observation.

It would be difficult by this method to map the distribution of the inhibitory effectiveness in the entire region surrounding an ommatidium. Therefore, in another experiment we modified our method, utilizing the directional sensitivity and small visual fields of the ommatidia (*cf.* section on *Material*) to localize the stimulation. Instead of focussing spots of light on restricted regions of the eye, sources of light were presented in different positions in front of the eye. Although these sources illuminated the whole eye, they stimulated only those ommatidia in whose visual fields they were located. One very small source, for exciting the ommatidium from which responses were recorded, was the filament of a bare ophthalmoscope bulb mounted 6 cm. in front of the eye on the turntable that carried the preparation: its position, once having been adjusted to yield the maximum discharge of impulses, thereafter remained fixed with respect to the eye. The second light source, for inhibiting the activity of this ommatidium, was furnished by the main optical system. The objective lens ordinarily used was removed, resulting in the formation of a virtual source of light 2 cm. in diameter, located 30 cm. in front of the eye. This source could be made to appear in any desired position in the visual field of the eye by rotating the horizontal turntable carrying the preparation and by rotating in a vertical plane the mirror system that directed the light onto the eye. The eye was located at the intersection of the axes of rotation of the turntable and mirror system; protractors centered on each axis permitted the angular coordinates of the source to be specified with respect to arbitrary reference planes through the eye.

This arrangement enabled us to determine contours on a map of the visual field of the eye for several intensities of inhibiting illumination, giving the

angular positions of the virtual source at which a certain constant inhibitory effect was exerted upon the selected ommatidium. The criterion effect was arbitrarily chosen to be a transient reduction of the frequency of discharge by just one-half, measured at the minimum frequency in the initial depression immediately following the onset of the inhibiting light. (Fig. 4, "—2.0" shows an effect of approximately this magnitude.) In making these determinations the ommatidium was excited by steady illumination for several minutes at a time; after the discharge of impulses had reached a steady rate, the inhibiting light was flashed on for exposures of 1 second duration at intervals of 4 seconds. Setting the intensity of the inhibiting light at a fixed value, we then adjusted its position in the visual field by rotating the mirror system or the turntable, or both, until the criterion effect was obtained. This position, read from the protractors, gave one point on a contour for that particular intensity. The preparation was then allowed to rest for several minutes (exciting light turned off), and the process repeated to determine another point on this same contour. Contours obtained for two values of inhibiting intensity are shown in Fig. 7. These results are typical of those found in other similar but less extensive experiments.

These measurements of inhibitory effectiveness of a light in various positions in the visual field were compared with the results of the previous experiment in which a spot of light was projected directly upon the surface of the eye in various locations. To do this, an approximate correlation was made between the angular position of the inhibiting light source in the visual field of the eye and the location on the surface of the eye of the group of ommatidia it stimulated. This correlation was made by noting the location of the pseudo-pupil<sup>2</sup> seen from different directions of view. The correlation was then used to transform the contours on the map of the visual field into contours on a map of the surface of the eye (insert of Fig. 7). Although different methods were used, the conclusions to be drawn from the two experiments are in essential agreement: in each case, the activity of the selected ommatidium could be inhibited by the stimulation of other ommatidia separated from it by as much as several millimeters; the inhibition diminished with increasing distance, and the diminution was more rapid in the dorso-ventral direction than in the antero-posterior.

Thus the activity of any one ommatidium is inhibited by illumination

<sup>2</sup> The pseudo-pupil is a black spot usually about 2 mm. in diameter, that is observed in a fresh eye; it is caused by the failure of those ommatidia that are pointed in the direction of the observer to reflect to his eye any light that falls on them. Light from a given direction can penetrate and stimulate efficiently only those ommatidia that lie within the confines of the pseudo-pupil that is observed from that direction. The size of the pseudo-pupil varies considerably from one part of the eye to another; consequently the stimulus conditions in the two experiments that are being discussed in this section were not strictly comparable.

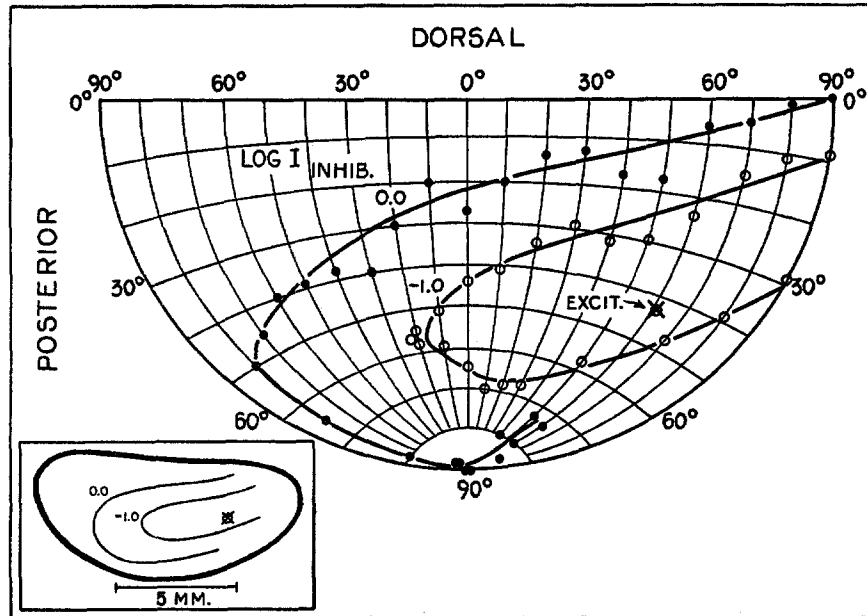


FIG. 7. Map showing the distribution of inhibitory effectiveness, with respect to a particular ommatidium, of light in the visual field of the eye. Contours are plotted for two intensities of a light source giving the locations at which it produced a constant inhibitory effect upon the ommatidium. A small incandescent lamp was mounted in front of the eye at the position (labelled "Excit.") that produced the most effective excitation of the particular ommatidium selected. The light source used to produce the inhibitory effect was tested in various positions in front of the eye to determine those positions at which it produced a certain constant magnitude of inhibition of the discharge in the optic nerve fiber from the ommatidium (transient reduction of the steady frequency to one-half its value, measured at the minimum frequency in the initial depression immediately following the onset of the inhibiting light). Each of the positions thus determined furnished one point on the contour corresponding to the particular value of inhibiting intensity employed. The highest intensity employed ( $\log I_{\text{inhib.}} = 0.0$ ) illuminated the surface of the eye with approximately 0.1 lumen/cm.<sup>2</sup>; points indicated by solid circles. For the points indicated by open circles the intensity was one-tenth this value ( $\log I_{\text{inhib.}} = -1.0$ ). The source was circular; its diameter subtended a visual angle of 4°. In this map (a globular projection representing approximately one-half of the total visual field of the eye) the plane of the equator is inclined approximately 20° above the horizontal plane of the animal's body; the 0° meridian lies approximately in a transverse plane.

The insert, lower left, is a sketch of the eye (heavy outline) showing contours drawn on its surface corresponding to the contours shown in the main figure (see text).



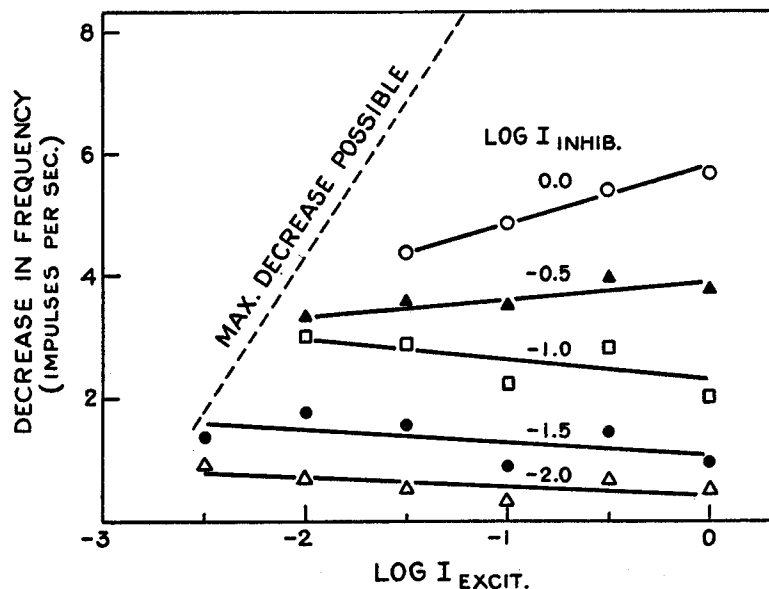


FIG. 8. Relation between the intensity of the exciting light illuminating an ommatidium and the magnitude of the inhibition exerted upon that ommatidium as a result of illuminating a nearby region of the eye at fixed intensity, for five different values of such inhibiting intensity. The abscissae give the intensities of the spot of light (0.25 mm. diameter) focussed on the ommatidium to excite a steady discharge of impulses in its optic nerve fiber ( $\log I_{\text{excit.}} = 0.0$  corresponds to an illumination of approximately 0.5 lumen/cm.<sup>2</sup>). The ordinates give the depression of frequency averaged over a 3 second period of inhibiting illumination, starting 3 seconds after the onset of the exciting light. This depression was measured by subtracting the value of the frequency during the exposure to inhibiting illumination from its value during similar control periods, when no inhibiting light was shining. Each point was determined from two experimental periods and three control periods. The region of the eye that was illuminated to produce the inhibition was 1 mm. in diameter, centered approximately 1 mm. from the ommatidium from which the responses were recorded. Each graph is for a fixed intensity of the inhibiting light (numbers give the logarithms of the relative intensities;  $\log I_{\text{inhib.}} = 0.0$  corresponds to an illumination of approximately 0.5 lumen/cm.<sup>2</sup> on the surface of the eye).

The dotted line (points omitted) labelled "Max. decrease possible" is a plot, to the same coordinates, of the frequency of the discharge (impulses per second) from the ommatidium as a function of the intensity of its exciting light. It is the locus that points would have if there had been inhibition sufficient to reduce the frequency of the discharge to zero.

anywhere within a region that covers an extensive area of the eye. Conversely, illumination of any given region of an eye inhibits the activity of many ommatidia in its vicinity, for the distributions of inhibitory effectiveness for most of the ommatidia of an eye overlap one another considerably.

(d) *Effect of Level of Excitation.*—If a fixed region of the eye is illuminated at a constant intensity, the frequency of discharge of a nearby ommatidium is depressed by an amount that is approximately constant irrespective of the level of excitation of the ommatidium.

We have performed several experiments in which we varied the intensity of the light exciting an ommatidium from which the optic nerve discharge was being recorded, while holding constant the intensity, area, and location of an inhibiting spot of light on a nearby region of the eye. The results of the most extensive of these experiments are shown in Fig. 8. The inhibitory effect was measured by the reduction in the frequency of discharge averaged over the entire period (3 seconds) of exposure to inhibiting light. It has been plotted as a function of the intensity of the light exciting the ommatidium, for five different intensities of inhibiting illumination. At all intensities of the inhibiting illumination, the reduction in frequency that was produced changed by only a small amount with change in the level of excitation of the receptor unit. For the two highest intensities of the inhibiting light, the depression of frequency became somewhat greater as the exciting intensity was increased, but we have not found this in other experiments. On the other hand the slight negative slope shown by each of the three lower lines has been observed in several other experiments; it probably resulted from the mutual interaction between that ommatidium from which activity was recorded and those in the region illuminated by the inhibiting beam. These deviations from constancy, it should be emphasized, were not very great, compared to the changes that were produced by varying the intensity of the inhibiting light over an equal range. To a first approximation, the reduction of the frequency of discharge from an ommatidium caused by a constant inhibitory stimulus in its neighborhood has been found to be independent of its level of excitation.

#### DISCUSSION

Our study has shown that the lateral eye of *Limulus* is more than an aggregation of independent receptors; it has a simple functional organization. Activity in any one optic nerve fiber, although it can be elicited by illumination of only the one specific receptor unit (ommatidium) from which the fiber arises, nevertheless may be affected by illumination of other ommatidia of the eye. The influence exerted upon a receptor unit by the activity of its neighbors appears to be purely inhibitory; it is of interest to consider

how this action may take place and what its role may be in the physiology of vision.

Our present investigations provide a first step in the analysis of the mechanism of inhibition in the eye of *Limulus*, and suggest the direction that future studies may take. Some possible mechanisms can be eliminated immediately.

Activity of receptor units might release chemical agents, which on diffusion through the tissues of the eye could affect the responses of neighboring elements. The inhibitory action, however, is so rapid that this seems out of the question; slowing of the discharge of a receptor unit begins suddenly in a few tenths of a second following illumination of a region of the eye several millimeters distant. Moreover, preliminary measurements that we have made show that the latency of the inhibition does not change markedly with changing the distance from the inhibiting area to the affected receptor element: transmission time for the effect seems to be only a small fraction of the latent period.

Electric currents generated by the retinal action potentials of receptors and flowing in the volume-conducting mass of the eye tissue could presumably affect the activity of nearby retinal elements. However, it is unlikely that the inhibition we are dealing with is produced in this way, for, in *Limulus*, the retinal action potentials that are recorded by electrodes in tissue external to the receptor elements are small and are transient, subsiding almost to zero in the first few tenths of a second of steady illumination (Hartline, Wagner, and MacNichol, 1952). The inhibition, we have shown, although exhibiting an initial maximum, remains quite strong for many seconds, as long as the inhibitory light continues to shine. Moreover, isolation of the nerve strand by dissection up to its ommatidium was shown to abolish the inhibitory effect, although the preparation was always kept immersed in blood (or sea water). Such dissection could hardly have altered, to any great extent, the gross electrical current flow in the volume-conducting medium.

The inhibitory effect apparently depends on the integrity of the nervous interconnections in the plexus of fibers just behind the layer of ommatidia. It would appear, then, that the search for an explanation of the inhibitory action can be narrowed to an investigation of the nature of the influence transmitted in the plexus and its mode of action on the sensory discharge.

Histological studies of the *Limulus* eye have failed to show any ganglion cells within the plexus, and functional studies have shown that nerve impulses can traverse the fiber pathways from ommatidia to optic nerve in either direction, apparently without interference. Still, some neural relay in the plexus may have escaped notice, and the inhibitory influence might be exerted upon it, or in some other way modify the sensory discharge as it

traverses the plexus. However, this could not explain the slowing of the rate of discharge that was observed when the impulses were recorded by a micro-electrode within an ommatidium. Evidently the inhibitory action is exerted within the ommatidium itself, where the conducted impulses originate. This direct action upon an ommatidium does not operate by inducing some reaction within it that interferes with the access of light to the sensory structure (as by some unknown pigment migration or other retinomechanical effect). This is shown by the inhibition exerted upon an after-discharge or other form of activity that may occur in darkness.

We have been unsuccessful as yet in attempts to record impulses in the cross-connections of the plexus or to demonstrate nervous activity, clearly associated with inhibitory effects, in fibers that run between the ommatidia in the plexus. However, we have noted the occurrence of prominent electrotonic potentials in fiber bundles in the *Limulus* eye (cf. Hartline, Wagner and MacNichol, 1952), and these may be significant in the inhibitory process.

A search for slow potential changes that might be recorded by microelectrodes in the ommatidia during inhibition, measurement of the changes in threshold for electrical stimulation of the ommatidia, exploration of the inhibiting effects produced by antidromic stimulation of the optic nerve (cf. Hartline, Wagner, and Tomita, 1953), and a survey of the action of pharmacological agents<sup>3</sup> are among the lines being pursued in further studies to elucidate the mechanism of the inhibitory effect.

Interaction in the eye is an example of an integrative process in a sensory system that takes place in the receptor organ itself. Mutual inhibition among the receptor units in the eye of *Limulus* is an important factor in determining the over-all patterns of nervous activity in the visual pathways of this animal. The discharge of impulses in any one optic nerve fiber, in response to steady illumination of the eye, depends not only upon the stimulus to the specific receptor unit from which that nerve fiber arises but also upon the spatial distribution of the stimulation over the entire population of mutually interacting elements. Furthermore, when the illumination changes, the transient component of the inhibitory influence modifies the temporal patterns of the responses of the individual receptor units. Thus a relatively simple, purely inhibitory interaction results in the generation of patterns of activity in the optic tract that are more than mere copies of the spatial and temporal patterns of stimulation on the sensory mosaic. In the vertebrate retina interaction has even more complex effects, for it comprises both excitatory and inhibitory influences. Indeed, the diversity of the responses of various optic nerve fibers in the vertebrate eye is probably the result of a complex interplay

<sup>3</sup>Dr. E. F. MacNichol (personal communication) found that 5 per cent ethyl alcohol in sea water reversibly abolished the inhibitory effect in the *Limulus* eye, without affecting the responses to light to any great extent.

of excitatory and inhibitory components of interaction in the retina (Hartline, 1941-42; Kuffler, 1953; Granit, 1952, and 1955, chapter 2, section 5).

The inhibitory component of the interaction in the vertebrate retina<sup>4</sup> is similar, in many respects, to the purely inhibitory interaction that we have found in the eye of *Limulus*. In the frog (Hartline, 1939) and in the cat (Kuffler, 1953) it has been shown that illumination of some parts of the receptive field of a retinal ganglion cell may inhibit responses generated by illuminating other parts of the same receptive field. Even more analogous to our observations on *Limulus* is Barlow's finding (1953) that the responses of a retinal ganglion cell in the frog may be inhibited by illumination of retinal regions entirely outside of its receptive field. (Recently one of us, HGW, has confirmed this observation.)

Inhibitory interaction by itself can achieve important visual effects. One of its consequences is the enhancement of visual contrast. In an animal's normal environment different receptors of the eye are usually subjected to unequal intensities of illumination from different parts of the visual field. In *Limulus* we have shown that the more intensely illuminated receptor units exert a stronger inhibition upon the less intensely illuminated units than the latter exert upon the former, especially if they are close together. As a result, differences in activity from differently lighted retinal regions are exaggerated; thus the contrast is enhanced (Hartline, 1949). In human vision, many of the familiar properties of simultaneous brightness contrast can be explained by postulating a similar inhibitory interaction in the visual pathways (Fry, 1948); if the inhibitory influence is assumed to decrease with increasing distance between retinal regions, border contrast and related effects may find an explanation.

#### SUMMARY

In the compound lateral eye of *Limulus* each ommatidium functions as a single receptor unit in the discharge of impulses in the optic nerve. Impulses originate in the eccentric cell of each ommatidium and are conducted in its axon, which runs without interruption through an extensive plexus of nerve fibers to become a fiber of the optic nerve. The plexus makes interconnections among the ommatidia, but its exact organization is not understood.

The ability of an ommatidium to discharge impulses in the axon of its eccentric cell is reduced by illumination of other ommatidia in its neighborhood: the threshold to light is raised, the number of impulses discharged in response to a suprathreshold flash of light is diminished, and the frequency

<sup>4</sup>In the auditory system of the cat, Galambos and Davis (1944) observed that the activity of single elements could be inhibited by tones that presumably excited other parts of the organ of Corti than those concerned in the excitation of the elements in question.

with which impulses are discharged during steady illumination is decreased. Also, the activity that can be elicited under certain conditions when an ommatidium is in darkness can be inhibited similarly. There is no evidence for the spread of excitatory influences in the eye of *Limulus*.

The inhibitory influence exerted upon an ommatidium that is discharging impulses at a steady rate begins, shortly after the onset of the illumination on neighboring ommatidia, with a sudden deep minimum in the frequency of discharge. After partial recovery, the frequency is maintained at a depressed level until the illumination on the neighboring receptors is turned off, following which there is prompt, though not instantaneous recovery to the original frequency.

The inhibition is exerted directly upon the sensitive structure within the ommatidium: it has been observed when the impulses were recorded by a microelectrode thrust into an ommatidium, as well as when they were recorded more proximally in single fibers dissected from the optic nerve.

Receptor units of the eye often inhibit one another mutually. This has been observed by recording the activity of two optic nerve fibers simultaneously.

The mediation of the inhibitory influence appears to depend upon the integrity of nervous interconnections in the plexus: cutting the lateral connections to an ommatidium abolishes the inhibition exerted upon it. The nature of the influence that is mediated by the plexus and the mechanism whereby it exerts its inhibitory action on the receptor units are not known.

The depression of the frequency of the discharge of nerve impulses from an ommatidium increases approximately linearly with the logarithm of the intensity of illumination on receptors in its vicinity.

Inhibition of the discharge from an ommatidium is greater the larger the area of the eye illuminated in its vicinity. However, equal increments of area become less effective as the total area is increased.

The response of an ommatidium is most effectively inhibited by the illumination of ommatidia that are close to it; the effectiveness diminishes with increasing distance, but may extend for several millimeters.

Illumination of a fixed region of the eye at constant intensity produces a depression of the frequency of discharge of impulses from a nearby ommatidium that is approximately constant, irrespective of the level of excitation of the ommatidium.

The inhibitory interaction in the eye of *Limulus* is an integrative process that is important in determining the patterns of nervous activity in the visual system. It is analogous to the inhibitory component of the interaction that takes place in the vertebrate retina. Inhibitory interaction results in the exaggeration of differences in sensory activity from different regions of the eye illuminated at different intensities, thus enhancing visual contrast.

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